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09/988929

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L1: Entry 1 of 1 File: USPT Aug 8, 2000

US-PAT-NO: 6100091
DOCUMENT-IDENTIFIER: US 6100091 A

5,888,790

TITLE: Modified acyl-ACP desaturase

DATE-ISSUED: August 8, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
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| Shanklin; John | Shoreham | NY | | |
| Lindgvist; Ylva | Jarfalla | | | SE |
| Schneider; Gunter | Jarfalla | | | SE |

ASSIGNEE-INFORMATION:

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| Brookhaven Science Associates | Upton | NY | | | 02 |

APPL-NO: 09/ 276295 [PALM]
DATE FILED: March 25, 1999

PARENT-CASE:
RELATED APPLICATIONS The subject patent application is a continuation of U.S. application Ser. No. 08/853,979, filed May 9, 1997, now U.S. Pat. No. 5,888,790, which was a continuation-in-part of U.S. application Ser. No. 08/689,823, filed Aug. 14, 1996, now U.S. Pat. No. 5,705,391, the contents of which are incorporated herein by reference.

INT-CL: [07] C12 N 15/09, C12 N 9/02, C12 N 15/29, C12 N 5/14, C07 H 21/04

US-CL-ISSUED: 435/455; 435/189, 435/252.3, 435/254.11, 435/320.1, 435/325, 435/410, 435/440, 536/23.2
US-CL-CURRENT: 435/455; 435/189, 435/252.3, 435/254.11, 435/320.1, 435/325, 435/410, 435/440, 536/23.2

FIELD-OF-SEARCH: 435/189, 435/252.3, 435/254.11, 435/320.1, 435/325, 435/410, 435/440, 435/455, 536/23.2

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

| | PAT-NO | ISSUE-DATE | PATENTEE-NAME | US-CL |
|---|---|---|---|---|
| ☐ | 5705391 | January 1998 | Cahoon et al. | 435/419 |
| ☐ | 5888790 | March 1999 | Cahoon et al. | 435/172.3 |

ART-UNIT: 163

PRIMARY-EXAMINER: Wax; Robert A.

ASSISTANT-EXAMINER: Stole; Einar

ABSTRACT:

Disclosed is a methods for modifying the chain length and double bond positional specificities of a soluble plant fatty acid desaturase. More specifically, the method involves modifying amino acid contact residues in the substrate binding channel of the soluble fatty acid desaturase which contact the fatty acid. Specifically disclosed is the modification of an acyl-ACP desaturase. Amino acid contact residues which lie within the substrate binding channel are identified, and subsequently replaced with different residues to effect the modification of activity.

58 Claims, 2 Drawing figures

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**End of Result Set**

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L1: Entry 1 of 1 File: USPT Aug 8, 2000

US-PAT-NO: 6100091
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TITLE: Modified acyl-ACP desaturase

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| Lindgvist; Ylva | Jarfalla | | | SE |
| Schneider; Gunter | Jarfalla | | | SE |

US-CL-CURRENT: 435/455; 435/189, 435/252.3, 435/254.11, 435/320.1, 435/325, 435/410, 435/440, 536/23.2

CLAIMS:

We claim:

1. A method for modifying the chain length and double bond positional specificities of a soluble plant fatty acid desaturase, the method comprising modifying one or more amino acid residues in the substrates binding channel of the soluble plant fatty acid desaturase which do not make direct contact with substrate.

2. A method for modifying the chainlength and double bond specificities of a soluble plant fatty acid desaturase, the method comprising modifying the amino acid residue corresponding to amino acid 200 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

3. The method of claim 1 wherein the amino acid residues are located at the upper part of the substrate binding channel of the soluble fatty acid desaturase.

4. The method of claim 3 wherein the soluble plant fatty acid desaturase is an acyl-ACP desaturase.

5. The method of claim 4 wherein the acyl-ACP desaturase is a .DELTA..sup.9 desaturase.

6. The method of claim 5 wherein the .DELTA..sup.9 desaturase is produced by a plant selected from the group consisting of Thunbergia alata or Ricinus communis.

7. The method of claim 6 wherein the amino acid residues are selected from the group consisting of residues corresponding to amino acids 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

8. A method for modifying the chain length and double bond positional specificities of an acyl-ACP desaturase, comprising:

a) providing the primary amino acid sequence of the acyl-ACP desaturase;

b) aligning the primary amino acid sequence of the acyl-ACP desaturase with the primary amino acid sequence of the Ricinus communes .DELTA..sup.9 desaturase for maximum sequence conservation;

c) constructing a 3-dimensional model for the acyl-ACP desaturase based on the sequence conservation with the Ricinus communis .DELTA..sup.9 desaturase;

d) identifying amino acid residues which most closely correspond to amino acids 200, 203, 204, 205, 206, and 207 of the Ricinus communis .DELTA..sup.9 desaturase, of the structure modeled in step c); and

e) generating a mutant acyl-ACP desaturase having modified chain length and double bond positional specificities by replacing one or more of the amino acid residues identified in step d) with another amino acid residue.

9. A mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the mutant containing a point mutation at one or more amino acid residues which do not make direct contact with substrate.

10. The mutant acyl-ACP desaturase of claim 9 wherein the first fatty acid has a chain-length of 16:0 and the second fatty acid has a chain-length of 18:0.

11. The mutant acyl-ACP desaturase of claim 9 wherein the amino acid residues which do not make direct contact with substrate are selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 desaturase.

12. A mutant acyl-ACP desaturase having one or more amino acid substitutions at residues which do not make direct contact with substrate, which is characterized by changes in chain-length and double bond positional specificity as compared to the wild-type acyl-ACP desaturase counterpart.

13. The mutant acyl-ACP desaturase of claim 12 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the residue corresponds to amino acid 200 of the Ricinus communis .DELTA..sup.9 desaturase.

14. The mutant acyl-ACP desaturase of claim 12 wherein the residues are located at the upper part of the substrate binding channel.

15. The mutant acyl-ACP desaturase of claim 14 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the residues are selected from the group consisting of residues corresponding to amino acids 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.2 ACP desaturase.

16. The mutant of claim 13 or claim 15 wherein the .DELTA..sup.9 acyl-ACP desaturase is produced by mutagenizing nucleic acid cloned from Thunbergia alata or Ricinus communis.

17. A nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at one or

more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

18. The nucleic acid sequence of claim 17 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at the residue corresponding to reside 200 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

19. The nucleic acid sequence of claim 17 wherein the amino acid residue is located at the upper part of the substrate binding channel of the acyl-ACP desaturase.

20. The nucleic acid sequence of claim 19 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid residues selected from the group consisting of residues corresponding to amino acids 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

21. A DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at one or more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

22. The DNA expression construct of claim 21 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at the residue corresponding to residue 200 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

23. The DNA expression construct of claim 21 wherein the amino acid residue is located at the upper part of the substrate binding channel of the acyl-ACP desaturase.

24. The DNA expression construct of claim 23 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid residues selected from the group consisting of residues corresponding to residue 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

25. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at one or more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

26. The cell of claim 25 wherein the point mutation is introduced into

wild-type Ricinus communis .DELTA..sup.9 desaturase at the amino acid residue corresponding to residue 200 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

27. The cell of claim 25 wherein the amino acid residue is located at the upper part of the substrate binding channel of the acyl-ACP desaturase.

28. The cell of claim 27 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid residues selected from the group consisting of residues corresponding to residue 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

29. The cell of claim 25 which is a prokaryotic cell.

30. The cell of claim 25 which is a eukaryotic cell.

31. The cell of claim 30 which is a plant cell.

32. A DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a chimeric acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold.

33. The DNA expression construct of claim 32 wherein the chimeric acyl-ACP desaturase comprises .DELTA..sup.6 -16:0 in which amino acids corresponding to amino acids 172-201 of Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 178-207 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase from a .DELTA..sup.9 -18:0 ACP desaturase.

34. The DNA expression construct of claim 32 wherein the chimeric acyl-ACP desaturase comprises .DELTA..sup.6 -16:0 in which amino acids corresponding to amino acids 172-196 of Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 178-202 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase from a .DELTA..sup.9 -18:0 ACP desaturase.

35. The DNA expression construct of claim 32 wherein the chimeric acyl-ACP desaturase comprises a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176, 183, 184, 200, 201 and 202 of the Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to 181, 188, 189, 205, 206 and 207 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

36. The DNA expression construct of claim 32 wherein the chimeric acyl-ACP desaturase comprises a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids 183 and 184 of the Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 188 and 189 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

37. The DNA expression construct of claim 35 wherein the chimeric acyl-ACP desaturase comprises a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176 and 195 of the Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to 181 and 200 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

38. The DNA expression construct of claim 35 wherein the chimeric acyl-ACP

desaturase comprises a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176, 195, 200, 201 and 202 of the Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 181, 200, 205, 206 and 207 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

39. A method for modifying the chain length and double bond positional specificities of a soluble plant fatty acid desaturase, the method comprising modifying one or more amino acid contact residues in the substrate binding channel of the soluble fatty acid desaturase which contact the fatty acid, and modifying one or more amino acids which do not contact substrate.

40. The method of claim 39 wherein the soluble plant fatty acid desaturase is an acyl-ACP desaturase.

41. The method of claim 40 wherein the acyl-ACP desaturase is a .DELTA..sup.9 desaturase.

42. The method of claim 41 wherein the .DELTA..sup.9 desaturase is produced by a plant selected from the group consisting of Thunbergia alata or Ricinus communis.

43. The method of claim 42 wherein the amino acid contact residues are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase and the amino acids which do not contact substrate are selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206, and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

44. A method for modifying the chain length and double bond positional specificities of an acyl-ACP desaturase, comprising:

a) providing the primary amino acid sequence of the acyl-ACP desaturase;

b) aligning the primary amino acid sequence of the acyl-ACP desaturase with the primary amino acid sequence of the Ricinus communis .DELTA..sup.9 desaturase for maximum sequence conservation;

c) constructing a 3-dimensional model for the acyl-ACP desaturase based on the sequence conservation with the Ricinus communis .DELTA..sup.9 desaturase;

d) identifying amino acid contact residues within the substrate binding channel of the structure modeled in step c);

e) identifying amino acid residues which most closely correspond to the amino acids 200, 203, 204, 205, 206, and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase of the structure modeled in step c); and

f) generating a mutant acyl-ACP desaturase having modified chain length and double bond positional specificities by replacing one or more of the amino acid contact residues identified in step d) with another amino acid residue, and replacing one or more of the amino acid residues identified in step e) with another amino acid residue.

45. A mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold which contains a point mutation at one or more amino acid contact residues in the substrate binding channel and also contains a point mutation at one or more amino acid residues which do not make direct contact with substrate.

46. The mutant acyl-ACP desaturase of claim 45 wherein the first fatty acid has a chain-length of 16:0 and the second fatty acid has a chain-length of 18:0.

47. A mutant acyl-ACP desaturase having one or more amino acid substitutions at contact residues within the substrate binding channel and one or more amino acid substations at residues which does not make direct contact with substrate.

48. The mutant acyl-ACP desaturase of claim 47 which is characterized by changes in chain-length and double bond positional specificity as compared to the wild-type acyl-ACP desaturase counterpart.

49. The mutant acyl-ACP desaturase of claim 48 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the contact residues within the substrate binding channel are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase, and the residue which does not make direct contact with substrate is selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

50. The mutant of claim 49 wherein the .DELTA..sup.9 acyl-ACP desaturase is produced by mutagenizing nucleic acid cloned from Thunbergia alata or Ricinus conmmunis.

51. A nucleic acid sequence encoding a mutant acyl-ACP desaturase characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold which contains a point mutation at one or more amino acid contact residues in the substrate binding channel and further contains a point mutation at one or more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

52. The nucleic acid sequence of claim 51 wherein the first fatty acid has a chain-length of 16:0 and the second fatty acid has a chain-length of 18:0.

53. The nucleic acid sequence of claim 52 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the contact residues within the substrate binding channel are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase, and the residue which does not make direct contact with substrate is selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

54. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at one or more amino acid contact residues in the substrate binding channel, and one or more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence

alignment with the Ricinus communis .DELTA..sup.9 desaturase.

55. The cell of claim 54 wherein the amino acid contact residues are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase and the amino acid residues which do not make direct contact with substrate are selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

56. The cell of claim 54 which is a prokaryotic cell.

57. The cell of claim 54 which is a eukaryotic cell.

58. The cell of claim 57 which is a plant cell.

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L2: Entry 4 of 5 File: USPT Mar 30, 1999

US-PAT-NO: 5888790
DOCUMENT-IDENTIFIER: US 5888790 A

TITLE: Modified Acyl-ACP desaturase

DATE-ISSUED: March 30, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Shanklin; John | Shoreham | NY | | |
| Lindqvist; Ylva | Jarfalla | | | SE |
| Schneider; Gunter | Jarfalla | | | SE |

US-CL-CURRENT: 435/440; 435/189

CLAIMS:

We claim:

1. A method for modifying the chain length and double bond positional specificities of a soluble plant fatty acid desaturase, the method comprising modifying amino acid contact residues in the substrate binding channel of the soluble fatty acid desaturase which contact the fatty acid.

2. The method of claim 1 wherein the soluble plant fatty acid desaturase is an acyl-ACP desaturase.

3. The method of claim 2 wherein the acyl-ACP desaturase is a .DELTA..sup.9 desaturase.

4. The method of claim 3 wherein the .DELTA..sup.9 desaturase is produced by a plant selected from the group consisting of Thunbergia alata or Ricinus communis.

5. The method of claim 4 wherein the amino acid contact residues are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Richinus communis .DELTA..sup.9 ACP desaturase.

6. A method for modifying the chain length and double bond positional specificities of an acyl-ACP desaturase, comprising:

a) providing the primary amino acid sequence of the acyl-ACP desaturase;

b) aligning the primary amino acid sequence of the acyl-ACP desaturase with the primary amino acid sequence of the Ricinus communis .DELTA..sup.9 desaturase for maximum sequence conservation;

c) constructing a 3-dimensional model for the acyl-ACP desaturase based on the sequence conservation with the Ricinus communis .DELTA..sup.9 desaturase;

d) identifying amino acid contact residues within the substrate binding channel of the structure modeled in step c); and

e) generating a mutant acyl-ACP desaturase having modified chain length and double bond positional specificities by replacing one or more of the amino acid contact residues identified in step d) with another amino acid residue.

7. A mutant acyl-ACP desaturase which is characterized by modification of one or more amino acid contact residues in the substrate binding channel and the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold.

8. The mutant acyl-ACP desaturase of claim 7 which contains a point mutation at an amino acid contact residue in the substrate binding channel.

9. The mutant acyl-ACP desaturase of claim 7 wherein the first fatty acid has a chain-length of 16:0 and the second fatty acid has a chain-length of 18:0.

10. A mutant acyl-ACP desaturase having an amino acid substitution at a contact residue within the substrate binding channel.

11. The mutant acyl-ACP desaturase of claim 10 which is characterized by changes in chain-length and double bond positional specificity as compared to the wild-type acyl-ACP desaturase counterpart.

12. The mutant acyl-ACP desaturase of claim 11 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the contact residues within the substrate binding channel are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

13. The mutant of claim 12 wherein the .DELTA..sup.9 acyl-ACP desaturase is produced by mutagenizing nucleic acid cloned from Thunbergia alata or Ricinus communis.

14. A chimeric acyl-ACP desaturase which is characterized by modification of one or more amino acid contact residues in the substrate binding channel and the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold.

15. The chimeric acyl-ACP desaturase of claim 14 comprising a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 172-201 of Ricinus communis .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 178-207 of the Ricinus communis .DELTA..sup.9 -18:0 ACP desaturase from a .DELTA..sup.9 -18:0 ACP desaturase.

16. The chimeric acyl-ACP desaturase of claim 14 comprising a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 172-196 of Ricinus communis .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 178-202 of the Ricinus communis .DELTA..sup.9 -18:0 ACP desaturase from a .DELTA..sup.9 -18:0 ACP desaturase.

17. The chimeric acyl-ACP desaturase of claim 14 comprising a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176, 183, 184, 200, 201 and 202 of the Ricinus communis .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 181, 188, 189, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

18. The chimeric acyl-ACP desaturase of claim 14 comprising a .DELTA..sup.6 16:0 ACP desaturase in which amino acids corresponding to amino acids 183 and 184 of the Ricinus communis .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 188 and 189 of the Ricinus communis .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

19. The chimeric acyl-ACP desaturase of claim 17 comprising a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176 and 195 of the Ricinus communis .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 181 and 200 of the Ricinus communis .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

20. The chimeric acyl-ACP desaturase of claim 17 comprising a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176, 195, 200, 201 and 202 of the Ricinus communis .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 181, 200, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

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**End of Result Set**

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L2: Entry 5 of 5 File: USPT Jan 6, 1998

US-PAT-NO: 5705391
DOCUMENT-IDENTIFIER: US 5705391 A

TITLE: Modified acyl-ACP desaturase

DATE-ISSUED: January 6, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Shanklin; John | Shoreham | NY | | |
| Lindgvist; Ylva | Jarfalla | | | SE |
| Schneider; Gunter | Jarfalla | | | SE |

US-CL-CURRENT: 435/419; 435/189, 435/243, 435/252.3, 435/254.11, 435/255.1, 435/320.1, 536/23.2

CLAIMS:

We claim:

1. A nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at an amino acid contact residue in the substrate binding channel, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

2. The nucleic acid sequence of claim 1 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

3. A DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterize by a point mutation at an amino acid contact residue in the substrate binding channel, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

4. The DNA expression construct of claim 3 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

5. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at an amino acid contact residue in the substrate binding channel, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

6. The cell of claim 5 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

7. The cell of claim 5 which is a prokaryotic cell.

8. The cell of claim 5 which is a eukaryotic cell.

9. The cell of claim 8 which is a plant cell.

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☐ 1. Document ID: US 6426447 B1

L2: Entry 1 of 5 File: USPT Jul 30, 2002

US-PAT-NO: 6426447
DOCUMENT-IDENTIFIER: US 6426447 B1

TITLE: Plant seed oils

DATE-ISSUED: July 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Knauf; Vic C. | Winters | CA | | |
| Thompson; Gregory A. | Davis | CA | | |

US-CL-CURRENT: 800/281; 435/419, 435/468, 800/298

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6100091 A

L2: Entry 2 of 5 File: USPT Aug 8, 2000

US-PAT-NO: 6100091
DOCUMENT-IDENTIFIER: US 6100091 A

TITLE: Modified acyl-ACP desaturase

DATE-ISSUED: August 8, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Cahoon; Edgar B. | Shoreham | NY | | |
| Shanklin; John | Shoreham | NY | | |
| Lindgvist; Ylva | Jarfalla | | | SE |
| Schneider; Gunter | Jarfalla | | | SE |

US-CL-CURRENT: 435/455; 435/189, 435/252.3, 435/254.11, 435/320.1, 435/325, 435/410, 435/440, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 3. Document ID: US 5981236 A

L2: Entry 3 of 5 File: USPT Nov 9, 1999

US-PAT-NO: 5981236
DOCUMENT-IDENTIFIER: US 5981236 A

TITLE: Geminivirus-based gene expression system

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Kridl; Jean | Davis | CA | | |
| Knauf; Vic C. | Winters | CA | | |
| Breuning; George | Davis | CA | | |

US-CL-CURRENT: 435/91.41; 435/320.1, 435/410, 435/419, 435/468, 435/91.4, 435/91.42

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw. Desc | Image

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☐ 4. Document ID: US 5888790 A

L2: Entry 4 of 5 File: USPT Mar 30, 1999

US-PAT-NO: 5888790
DOCUMENT-IDENTIFIER: US 5888790 A

TITLE: Modified Acyl-ACP desaturase

DATE-ISSUED: March 30, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Cahoon; Edgar B. | Shoreham | NY | | |
| Shanklin; John | Shoreham | NY | | |
| Lindqvist; Ylva | Jarfalla | | | SE |
| Schneider; Gunter | Jarfalla | | | SE |

US-CL-CURRENT: 435/440; 435/189

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw. Desc | Image

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☐ 5. Document ID: US 5705391 A

L2: Entry 5 of 5 File: USPT Jan 6, 1998

US-PAT-NO: 5705391
DOCUMENT-IDENTIFIER: US 5705391 A

TITLE: Modified acyl-ACP desaturase

DATE-ISSUED: January 6, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Cahoon; Edgar B. | Shoreham | NY | | |
| Shanklin; John | Shoreham | NY | | |
| Lindgvist; Ylva | Jarfalla | | | SE |
| Schneider; Gunter | Jarfalla | | | SE |

US-CL-CURRENT: 435/419; 435/189, 435/243, 435/252.3, 435/254.11, 435/255.1, 435/320.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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| acyl-ACP desaturase.clm. | 5 |

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L3: Entry 1 of 6 File: PGPB Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020150982
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020150982 A1

TITLE: Mutant fatty acid desaturase

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Shanklin, John | Shoreham | NY | US | |
| Cahoon, Edgar B. | Wilmington | DE | US | |

APPL-NO: 09/ 988929 [PALM]
DATE FILED: December 3, 2001

RELATED-US-APPL-DATA:
Application 09/988929 is a continuation-in-part-of US application 09/233856, filed January 19, 1999, ABANDONED

INT-CL: [07] C12 P 21/02, C12 N 1/21, C12 N 9/04, C07 H 21/04, C12 N 15/74, C12 P 7/64

US-CL-PUBLISHED: 435/69.1; 435/488, 435/252.33, 435/190, 435/320.1, 536/23.2, 435/134
US-CL-CURRENT: 435/69.1; 435/134, 435/190, 435/252.33, 435/320.1, 435/488, 536/23.2

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The present invention relates to a method for producing mutants of a fatty acid desaturase having a substantially increased activity towards fatty acid substrates with chains containing fewer than 18 carbons relative to an unmutagenized precursor desaturase having an 18 carbon atom chain length substrate specificity. The method involves inducing one or more mutations in the nucleic acid sequence encoding the precursor desaturase, transforming the mutated sequence into an unsaturated fatty acid auxotroph cell such as MH13 E. coli, culturing the cells in the absence of supplemental unsaturated fatty acids, thereby selecting for recipient cells which have received and which express a mutant fatty acid desaturase with an elevated specificity for fatty acid substrates having chain lengths of less than 18 carbon atoms. A variety of mutants having 16 or fewer carbon atom chain length substrate specificities are produced by this method. Mutant desaturases produced by this method can be introduced via expression vectors into prokaryotic and eukaryotic cells and can also be used in the production of transgenic plants which may be used to produce specific fatty acid products.

[0001] The present application is a continuation-in-part of U.S. patent application Ser. No. 09/233,856 filed on Jan. 19, 1999.

# WEST Search History

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| L3 | acyl-ACP desaturase.clm. | 6 | L3 |
| *DB=USPT; PLUR=YES; OP=ADJ* | | | |
| L2 | acyl-ACP desaturase.clm. | 5 | L2 |
| L1 | 6100091 | 1 | L1 |

END OF SEARCH HISTORY